76 Incidence of cartilage abnormalities on tracheal function in CFTR-deficient mice

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We have recently demonstrated that *Cftr* knockout ($Cftr^{-/-}$) and F508del-CFTR (ΔF/ΔF) mice exhibit structural abnormalities of the trachea characterized by disrupted or incomplete cartilage rings. Such defects may lead to upper or lower segmental constriction of the trachea. We, thus, investigated whether structural alterations in the tracheal area which contains sites of high CFTR expression, may contribute to tracheal and ventilatory dysfunction in cystic fibrosis (CF).
To test this hypothesis, we examined 1) the contractile response to carbachol (CCh) of the proximal and distal portions of trachea in $Cftr^{-/-}$ and ΔF/ΔF mice compared to their respective controls; 2) the tracheal relaxation in response to salbutamol of precontracted tracheal segments in $Cftr^{-/-}$ and $Cftr^{+/+}$ mice; 3) the pattern of breathing in $Cftr^{-/-}$ and $Cftr^{+/+}$ mice by using whole-body plethysmography.
Our data show in $Cftr^{-/-}$ mice a decreased contractile response to CCh in proximal trachea, and an impaired relaxation in response to salbutamol in both tracheal segments. In ΔF/ΔF mice, distal trachea was less sensitive to CCh than proximal trachea. Analysis of ventilation showed a lower breathing rate in $Cftr^{-/-}$ than in $Cftr^{+/+}$ mice due to a marked increase in both inspiratory and expiratory times.
We conclude that CFTR-deficient mice exhibiting abnormal tracheal architecture display tracheal dysfunction and altered respiratory airflow which may contribute to the pathogenesis of airway disease in CF patients.
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77* Regulation of the KCa3.1 channel by AMP-activated protein kinase in Cl^- secreting cells

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The vectorial transport of ions and water across epithelial cells depends on the co-ordination of the ion fluxes at the apical and basolateral membranes with energy supply. We provide the first evidence for a regulation by the 5′-AMP-activated protein kinase (AMPK) of the KCa3.1 channel expressed at the basolateral membrane of a large variety of epithelial cells. Co-immunoprecipitation and pull-down experiments showed that the C-terminal region of KCa3.1 (Leu345 to Ala400) interacts with the α1 and γ1 subunits of AMPK. AMPK-γ1 and KCa3.1 were also found to colocalized at the plasma membrane of HEK-293 cells. A decrease in KCa3.1 activity was observed in inside-out patch clamp experiments following the addition of AMP at a fixed ATP concentration (1 mM) with half inhibition at 140 μM. AMP-dependent inhibition was not observed in the presence of Ara-A (1 mM) an AMPK inhibitor. Coexpressing KCa3.1 in HEK-293 cells with the dominant negative mutant R299G of AMPK-γ1 resulted in an increase of the cells with a low response to AMP from 5% in control to 50% with the γ1 mutant. Finally, we show that the activation of AMPK by the membrane permeant AMPK activator AICAR causes a decrease of the KCa3.1-mediated short circuit currents in bronchial epithelial cell monolayers. The current decrease initiated by AICAR was reversed by coincubation with 10 μM Compound-C confirming a regulation of KCa3.1 by AMPK in a functional epithelium. Altogether our results point toward a metabolic control of KCa3.1 through AMPK activation.
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78 Expression of cystic fibrosis transmembrane regulator (CFTR) in human bone cells

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Introduction: Osteoporosis is a common and serious complication of cystic fibrosis (CF), and alteration of CFTR function in bone cells may contribute. Inactivation of the CFTR gene in mice causes severe osteopenia, and in adults with CF the ΔF508 mutation is an independent risk factor for low bone mineral density.
Methods: Osteoblastic cell lines (MG63 and SaOS), primary human osteoblasts and human osteoclasts cultured from peripheral blood mononuclear cells were examined for the expression of CFTR protein by Western blotting and immunofluorescence using a mouse anti-human CFTR (C-terminus) monoclonal antibody (clone 24).
Results: Primary human osteoblasts and osteoblastic cell lines were found to express mature (fully glycosylated 180 kD), ER-glycosylated (150 kD) and newly-synthesized non-glycosylated (130 kD) CFTR. Non-glycosylated and ER-glycosylated CFTR was detected in Western blots of osteoclast lysates, but minimal (<0.5% of total CFTR) fully glycosylated protein was found in these cells. Immunofluorescence suggested a cytoplasmic rather than membrane-associated distribution in all cell types examined.
Conclusion: CFTR protein is present in human osteoblasts and osteoclasts, although in the latter cell type the expression of mature, fully glycosylated CFTR is limited. The functional significance of these findings and their implication for CF-associated bone disease remain to be determined.
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79* Inflammation and abnormal tissue remodeling in F508del mutant mice

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Lung inflammation and airway remodeling are hallmarks of CF lung disease. We investigated transcriptional responses to epithelial injury in a mouse model of CF (F508del, Cftrtm1eur) using a quantitative PCR array of 56 genes differentially expressed after naphthalene injury of distal airways. Homozygous normal and mutant age matched littermates were treated in parallel with Naphthalene or carrier as control ($N = 4 \times 12$). In this gene cluster, significant ($P < 0.05$) over-expression of acute phase proteins (Orm1, Serpina3n, Ecm1, Lcn2) and metalloproteinases (Mmp8, Mmp9) was observed in unchallenged mutant animals compared to normal. Furthermore, several modulators of tissue repair were upregulated in mutant mice both before and after injury (Igfbp5, Il6st, Tnfrsf12, Egr1, Adam10, Adam17). After naphthalene injury, EGF receptor agonists Amphiregulin, Epiregulin and Heparin binding EGF were strongly induced in normal and mutant animals, correlating with an increase of major extracellular matrix (ECM) mRNA's (Co1a1, Col3a1, Elastin). Whereas expression levels were substantially reduced in normal animals seven days after injury, in CF mutant animals two to three fold higher levels were observed for these three genes at day seven ($P < 0.04$).
Conclusions: Our data support and extend the notion that inflammation and activation of tissue remodeling pathways occurs in unchallenged pathogen free F508del mice. This would explain the sustained ECM mRNA production in CF mouse lung after injury, suggesting an inherent tendency towards fibrosis in CF lungs, and providing new targets for therapy of irreversible lung damage in CF.
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